1. Introduction

In recent years, point-of-care testing (POCT) devices that can provide health monitoring in real-time and rapid diagnostic tests have received much attention\(^1\). However, blood sampling is still playing a major role in POCT although it causes pain and discomfort to patients\(^2\). Meanwhile, skin interstitial fluid (ISF) has become an attracting alternative to blood samples because ISF contains abundant biomarkers (e.g., glucose, cholesterol, and proteins) which can accurately reflect their concentrations in blood\(^3\). Therefore, it is required to develop a simple and minimally invasive approach to extract skin ISF for routine self-medical monitoring in daily preventive healthcare.

Microneedles (MNs), the micron-sized needles which can penetrate the dermis layer painlessly that were initially developed for transdermal drug delivery, have been recently reckoned as promising components to extract ISF. Porous MNs with interconnected micropores to absorb ISF by capillary effect have been developed recently. The absorbed ISF can be transported to an analysis system directly, and therefore biosensors can be combined for further real-time measurement. As materials, biocompatible, even biodegradable polymers have been investigated to form porous structures\(^4,5\).

In this research, we proposed a new approach to fabricate biodegradable porous MNs with emulsion droplets. Polylactic acid (PLA) microspheres prepared by single emulsion was used to form interconnected pores directly and a simple heat treatment was applied to bond microspheres together. The MN arrays after heat treatment indicated increased mechanical strength and rapid extraction of ISF through passive capillary action. The fast and simple fabrication of biodegradable porous MNs has therefore the potential for scale-up production.

2. Fabrication method

In our fabrication method, PLA microspheres were used to fabricate continuous pores in MNs for fluid transport. PLA microspheres were initially developed for controlled drug delivery systems due to their biocompatible and biodegradable properties\(^6\). As shown in Fig.1(a–c), 6.7 % (w/v) of PLA solution was prepared in dichloromethane (DCM) as organic phase. The organic phase was then blended into the water phases that contained 5 % (w/v) of polyvinyl alcohol (PVA) as a surfactant. The mixture was agitated with 1000 rpm at room temperature until DCM was evaporated, which results in PLA microspheres (b). PLA microspheres solution was then poured into the PDMS female mold and vacuumed (c). The entire mold was dried at 50 ℃ for 2 h in a convection oven, and peeled off for subsequent heat treatment (d).
microspheres only in PVA solution. Once PLA microspheres are formed, spherical shape can be maintained stable in ambient environment. The diameter of the fabricated microspheres was measured as $15.5 \pm 6.9 \mu m$. Subsequently, the PLA microsphere solution was poured into the PDMS female mold prepared from a metal master mold comprising of 169 pyramidal shaped microneedles of 1200 μm in length (Fig.1(d)). The vacuum was then applied to fill the microspheres into the cavities. Next, the entire mold was placed into a convection oven at 50 ℃ for 2 hours and the MN array was peeled off from the mold (Fig.1(e)). Finally, heat treatments at different temperatures (170, 180, 190, 200 ℃) were applied for 30 minutes to make microspheres melt and bond each other.

3. Result and evaluation

3.1 Shapes and dimensions of porous PLA MNs

The shapes and dimensions of porous PLA MNs were measured and demonstrated in Fig.2. After drying and peeling off from the mold, the height and the tip diameter of MNs were measured as $1120.6 \pm 47.4 \mu m$ and $33.1 \pm 14.6 \mu m$, respectively. The fabrication results revealed that shapes and sharp tips were maintained after heat treatments, whereas both the height and tip diameter of MNs shrunk slightly. It was considered that the shrinkage was caused by the melting and bonding of PLA microspheres inside MNs (Fig.2(a) and (b)).

3.2 Porous structure and porosity

The porous structure of the MNs was investigated using the scanning electron microscopy (SEM). Single MNs and cross-sectional images were shown in Table 1. After dried at 50 ℃ for 2 h, the continuous voids were formed, but the PLA microspheres were not bonded to each other. After heat treatment at 170 ℃ for 30 min, a portion of microspheres started to melt and bond together. It was considered that PLA spheres were started to melt as the melting point of used PLA was 170 ℃. With heating at 180 ℃ for 30 min, most of the microspheres were bonded altogether, which results in the formation of micron-sized interconnected pores finally. On the contrast, the microspheres were observed to overmelted and only a few interconnected voids were confirmed when PLA MNs were heated at 200 ℃.

<table>
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<tr>
<th>Table 1</th>
<th>SEM images of porous PLA MNs with heat treatment.</th>
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<td>MN</td>
<td>Cross-section</td>
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<td>Dried 50 ℃</td>
<td><img src="Fig.2_170%E2%84%83.jpg" alt="SEM image" /></td>
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<td>170 ℃ 30 min</td>
<td><img src="Fig.2_180%E2%84%83.jpg" alt="SEM image" /></td>
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<tr>
<td>180 ℃ 30 min</td>
<td><img src="Fig.2_190%E2%84%83.jpg" alt="SEM image" /></td>
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<td>200 ℃ 30 min</td>
<td><img src="Fig.2_200%E2%84%83.jpg" alt="SEM image" /></td>
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Next, the porosity of porous PLA MNs was measured by the water imbibition method using porous PLA films by comparing their mass before and after the fluid extraction. Firstly, the dry mass ($W_{dry}$) of the porous film was recorded. Then, the film was immersed into the deionized (DI) water, and the surface water was removed after the absorption was saturated. Then the mass was measured immediately, which was recorded as $W_{wet}$. The porosity was calculated using the following equation and the calculation results were charted (Fig.3).
Porosity(%) = \frac{W_{\text{wet}} - W_{\text{dry}}}{\rho_p} \times \frac{W_{\text{dry}}}{W_{\text{wet}}}

where \( \rho_p \) is the density of PLA (1.25 g/cm\(^3\)), and \( \rho_w \) is the density of the DI water (1.0 g/cm\(^3\)).

Without heat treatment, the porosity was measured as 27.2 ± 0.9%. As the temperature for heat treatment increased, the porosity decreased gradually because more PLA microspheres were melted and bonded, which results in the decrease of continuous voids inside the microneedles.

### 3.3 Absorbing ability

The absorption volume of sample fluids using the fabricated porous PLA MNs was investigated using 1% (w/w) of agarose gel covered with aluminum foil mimicking human skin \(^5\). A force of 5 N was applied onto the MN array to penetrate into the agarose gel. MNs with heat treatment at 170–200 °C successfully penetrated the aluminum foil and absorbed the sample fluid. However, the MNs that were dried at 50 °C could penetrate the aluminum foil but failed to maintain the MN structure with most of needles remained inside the agarose gel. The reason was considered to be the lack of adhesion among microspheres inside the MNs. The volume of sample fluid for continuous 1 minute and 2 minutes absorption is demonstrated in Fig.4. It was confirmed that the MNs heated at 180 °C absorbed the sample fluids the most in 2 min.

Subsequently, the extraction of glucose from the glucose-loaded agarose gel was evaluated. The agarose gel with 5 mM of glucose loaded in the ratio of 1% (w/w) was covered with an aluminum foil. The glucose-sensitive paper used for evaluations was prepared by pipetting the enzyme solution, containing glucose oxidase (GOx), peroxidase (HRP), and the chromogenic dye solution using tetramethylbenzidine (TMB), and dried at room temperature \(^5\). The sensing paper was then attached to the back side of the porous MN array (Fig.5). Next, pressure was applied by a finger to make MNs pierce the model skin. Once the glucose-loaded fluid was extracted and transported to the sensor layer, the reaction could be confirmed by the color change of TMB from GOx-catalyzed oxidation of glucose.

The results of sample ISF extraction and glucose sensing performance of porous PLA MNs are shown in Table 2. With the heat treatment above melting point of PLA, the extraction performance of MNs was improved significantly. Moreover, PLA MNs with heat treatment at 180 °C for 30 min extracted the sample fluid, which was transported to the sensor layer most rapidly. As a result, the glucose-sensitive paper was completely changed into blue color within 2 minutes. The reason for the little absorption of MNs without heat treatment was that the PLA microspheres were not bonded together to form stable interconnected pores for transporting sample fluid. On the contrary, the microspheres with heat treatment were considered to be melted and bonded to form the robust interconnected micropores and extract sample ISF by capillary effect.
3.4 Insertion test using porcine skin

In order to validate whether the porous PLA MNs were sufficiently rigid to puncture the skin, porcine skin was selected to mimic human skin for the insertion test since it is similar to the human skin structure comprised of stratum corneum, epidermis and dermis. Firstly, porous PLA MNs were inserted into the porcine skin by a finger pressure and peeled off from the skin. Subsequently, the porcine skin was stained with 1% (w/v) methylene blue for 15 min. Methylene blue remained on the skin was then wiped with ethanol and the penetration sites were examined using an optical microscope. As shown in Fig.6, porous PLA MNs with and without heat treatment could successfully puncture the porcine skin. However, while the MN patch without heat treatment was peeled off from the porcine after insertion, the whole structure of MN patch was not maintained and separated (Fig.6(a)). The reason was considered that the microspheres in the MN patch lacked adhesion to each other, and thus the patch was separated when peeled off from the porcine skin. On the contrary, most of the MNs with heat treatment at 180–200 °C successfully punctured the skin, which shows the effective penetration into the skin barrier (Fig.6(c–e)). At the same time, the structures of MNs were intact after peeling off the skin.

4. Conclusion

In this study, a simple and novel fabrication method of biodegradable porous MNs via emulsion droplets for rapid ISF extraction was proposed. PLA microspheres could be prepared by single emulsion and its spherical formation could be maintained stable in ambient temperature. After being applied with heat treatment, the microspheres were melted and bonded together to form interconnected microchannels to transport ISF continuously via capillary action. Furthermore, the effect of temperature of heat treatment was investigated. It was shown that the porous MNs showed rapidest extraction of sample ISF and effective skin penetration after heated at 180 °C for 30 min. It can be envisioned that mass production of biodegradable porous MNs can be achieved by the proposed fabrication method. Furthermore, we expect that minimally-invasive ISF extraction, biosensing as well as early diagnosis of diseases can be realized by integrating biosensors.

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References